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(71) Applicant *(for all designated States except US)*: **FERX INCORPORATED** [US/US]; 9171 Towne Centre Drive, Suite 575, San Diego, CA 92122-6218 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **TAPOLSKY, Gilles, Hugues** [FR/US]; 2460 Vassar Drive, Boulder, CO 80305 (US). **LI, Yuhua** [CN/US]; 901 Shavano Peak Drive, Superior, CO 80027 (US). **FAILING, Sarah, Nicole** [US/US]; 1301 E. 9th Avenue, #100, Boulder, CO 80218 (US). **RUDGE, Scott, Raymond** [US/US]; 656 Furman Way, Boulder, CO 80305 (US).

(74) Agent: **HUNT, Dale, C.**; KNOBBE, MARTENS, OLSON & BEAR, LLP, 2040 Main Street, 14th Floor, Irvine, CA 92614 (US).

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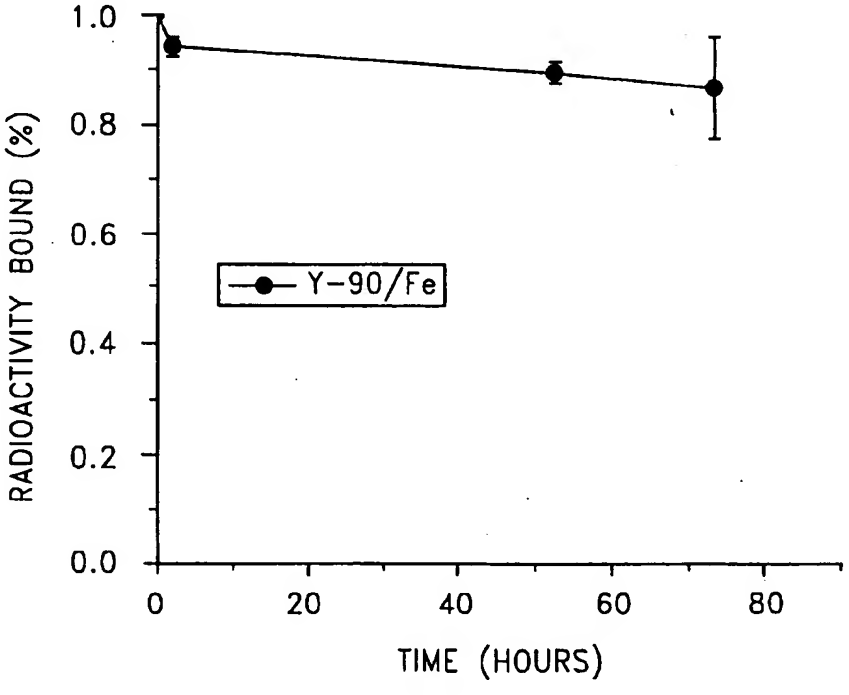

(57) Abstract: The invention relates to biodegradable, biocompatible magnetic particles useful for magnetic delivery of biologically active compounds for in vivo and ex vivo diagnostic and/or therapeutic activity. The particles exhibit excellent labeling efficiency and stability. They have an increased magnetic susceptibility as compared to previous compositions developed for magnetic delivery of diagnostics and therapeutics.

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MAGNETIC DELIVERY COMPOSITIONS

INTRODUCTION

[0001] The site-specific delivery of biologically active compounds has been a field of research for many years. One benefit of this type of delivery is the enhanced diagnostic and/or therapeutic activity of biologically active compounds such as chemotherapeutics, radionuclides, peptides, antibodies, and proteins with minimal systemic side effects. Many technologies have been developed with this in mind, yet efficiency is still limited, for example, by systemic side effects, lack of specific targeting, low targeting efficiency, redistribution to other organs as a function of time, lack of retention at the site of delivery, and low local drug concentration.

[0002] Targeted delivery systems have been developed based on antibodies or peptides linked to chemotherapeutic compounds, magnetic microparticles, magnetic liposomes, and regional therapy based on local injections. Also, particulate, implantable systems based on metallic or polymeric micro- or millimetric cylinders, biodegradable wafers, osmotic devices, and polymeric solutions forming a solid precipitate once injected parenterally have been used for a local treatment by local placement or injection.

[0003] Previous designs of particulate site-specific delivery technology either combined a targeting moiety with a carrier moiety, or relied on local injection of the therapeutic to the treatment site. As described in many publications, useful site-specific delivery technologies typically result from the combination of two components: a targeting moiety enabling precise localization, and a carrier moiety enabling the transport and delivery of a therapeutic and/or diagnostic amount of biologically active compounds to the site. Magnetic particle technology generally involves particle compositions including a magnetic targeting component integrated with a biologically active compound in a matrix of biocompatible material.

[0004] Serious shortcomings have made these technologies difficult to use in a clinical environment. For example, these technologies may not provide a mechanism for maintaining the microparticles at the desired sites, leading to inefficient concentrations of the biologically active compound at the targeted sites, and undesired redistribution over time. Other deficiencies include unstable preparations, low magnetic susceptibility, bioactive diffusion from the targeted site, lack of localization, and non-specific binding and toxicity to untargeted organs.

[0005] In addition, magnetic microparticle technologies are based on lipid, polymeric or other appropriate matrices that contain iron oxide (usually as magnetite or hematite) as the magnetic targeting material. The necessity of enclosing both the magnetic material and the biologically active compound(s) within a polymeric matrix or lipid vesicle is well known, as the presence of the matrix makes possible the delivery of increased quantities of the biologically active compound above that which could be accomplished by use of the iron oxide particles alone. The biologically

active compound(s) need to be released from the particles and vesicles by either diffusion through the matrix, degradation of the particle over time, or any other mechanism leading to the still active biological compound being at the desired site at sufficient concentrations for a sufficient period of time.

[0006] More recently, ferrocarbon particles have been used for targeted delivery. These particles comprise iron and carbon composite particles, wherein the iron provides magnetic susceptibility and the carbon carries the drug. The presence of carbon, even in low quantities, is required for delivering biologically active compounds. While these particles overcome previous deficiencies, as with other targeted delivery systems, both a targeting moiety and a carrier moiety are present within the particles. Until now, methods for associating biologically active compounds directly with the magnetic material were not known.

SUMMARY OF THE INVENTION

[0007] The present invention relates to a single component capable of carrying a biologically active compound in relevant quantities to a specific site. This involves magnetic particles having attached thereto (that is, precipitated, adsorbed, or labeled thereon) one or more biologically active compounds. The magnetic particles have the general properties of having Curie temperatures (Tc) greater than the normal human body temperature (37°C), having high magnetic saturation (> approximately 20 Am^2/kg), and being ferromagnetic or ferrimagnetic. Examples of the magnetic particles include iron oxides such as Jacobsite ($MnFe_2O_4$), Trevorite ($NiFe_2O_4$), iron sulfides such as Pyrrhotite (Fe_7S_8), and Greigite (Fe_4S_4), ceramics such as Alnico 5, Alnico 5 DG, Sm_2Co_{17}, $SmCo_5$ and NdFeB, as well as metals and alloys, such as iron (Fe), cobalt (Co), nickel (Ni), awaruite (Ni_3Fe) and wairauite (CoFe). Specifically excluded from the magnetic particles and the magnetically susceptible compositions of the instant invention are the iron oxides magnetite (Fe_3O_4), hematite (αFe_2O_3), and maghemite (γFe_2O_3). Each of the magnetic particles can have added to its chemical formula specific impurities that may or may not alter the magnetic propreties of the material. Doped ferromagnetic or ferrimagentic materials within the above limits of Curie temperatures and magnetic saturation values are considered to be within the scope of the instant invention. The term "metal particles" as used herein refers to paritcles formed from the metals and alloys listed above, while the term "metallic iron particles" refers to those formed from iron alone. Such particles are available commercially.

[0008] In that regard, examplary embodiments of the instant invention are listed in the following numbered paragraphs:

[0009] 1) A magnetically susceptible composition comprising a magnetic particle that has attached thereon a biologically active compound.

[0010] 2) Use of a magnetically susceptible composition for the manufacture of a medicament, wherein said composition comprises a magnetic particle having attached thereon a biologically active compound.

[0011] 3) Use of a magnetically susceptible composition for the treatment of disease in a patient in need thereof, wherein said composition comprises a magnetic particle having attached thereon a biologically active compound.

[0012] 4) Use of a magnetically susceptible composition for *in vivo* diagnostic imaging in a patient comprising:

[0013] 5) a) Establishing a magnetic field exterior to the body of said patient adjacent to the site to be imaged;

b) administering to said patient a magnetically responsive composition comprising a magnetic particle having attached thereon a biologically active compound;

c) producing an image based upon magnetic detection of said magnetically responsive composition; and

d) analyzing said image to provide a diagnosis.

[0014] 6) Use of a magnetically susceptible composition for *ex vivo* diagnostic imaging comprising:

a) providing a combination of a biological material and a magnetically susceptible composition that comprises a magnetic particle having attached thereon a biologically active compound;

b) applying a magnetic field to said combination; and

c) analyzing said biological material to provide a diagnosis.

[0015] 7) A kit for administering a biologically active substance comprising:

a) a first receptacle comprising a unit dose of magnetic particles; and

b) a second receptacle comprising a solution comprising one or more biologically active compounds.

[0016] 8) A method for local regional therapy comprising:

a) intra-arterial injection of a magnetically responsive composition comprising a magnetic particle having attached thereon a biologically active compound; and

b) establishment of an external magnetic field adjacent to a desired target region.

[0017] 9) A method for increasing the concentration of a biologically active compound at an *in vivo* site comprising:

a) injecting into a patient a magnetically responsive composition comprising a magnetic particle having attached thereon a biologically active compound; and

b) establishing an external magnetic field adjacent to the *in vivo* site where said increased concentration is desired.

[0018] 10) A process for producing a magnetically susceptible composition comprising attaching a biologically active compound onto a magnetic particle.

[0019] 11) A method for local regional therapy comprising:

a) intravenous injection of a magnetically responsive composition comprising a magnetic particle having attached thereon a biologically active compound; and

b) establishment of an external magnetic field adjacent to a desired target region.

[0020] 12) A composition made by the process comprising attaching a biologically active compound onto a magnetic iron particle.

[0021] 13) A magnetically susceptible composition comprising a magnetic iron particle having attached thereon a biologically active compound, whereby the composition is produced by a process comprising high-energy milling of said magnetic iron particle.

[0022] The direct attachment of a biologically active compound to magnetic particles enables exclusion of the carrier component from the particles, while still allowing for magnetically targeted particles that are biodegradable in body fluids over time.

[0023] Further examplary embodiments occur when the in any of the above embodiments the magentic particle is selected from the group consisting of iron, nickel, awaruite, wairauite, pyrrhotite, greigite, troilite, yttrium iron garnet, Alnico 5, Alnico 5 DG, Sm_2Co_{17}, $SmCo_5$ and NdFeB particles. Another examplary group of the above embodiments occurs when the magnetic particle of the embodiment is selected from magnetic particles composed of iron, nickel, awaruite, wairauite, pyrrhotite, greigite, troilite, and yttrium iron garnet particles. Yet another examplary embodiment of the above embodiments occurs when the magnetic particle is an iron particle. Yet another examplary embodiment of the above embodiments occurs when the magnetic particle is a wairauite (CoFe) particle. Another exemplary group of the above embodiments occurs when the magnetic particles are those that have a magnetic saturation value of approximately 100 Am^2/kg. Yet another exemplary group of of the above embodiments occurs when the magnetic particle of any of the above embodiments is ferrimagnetic.

[0024] While it is relatively straightforward to attach such compounds onto iron oxide particles, composite particles of iron and carbon, carbon particles alone, particles of high surface area such as alumina or silica, or particles with anionic groups such as hydroxyapatite, it was unexpected that synthetic, processed, or raw magnetic particles could be used directly to deliver relevant therapeutic quantities of biologically active compounds. Furthermore, particles using high surface area materials (for example, activated carbon, alumina, silica, or hydroxyapatite) depend upon the relatively high surface area, pore size and pore distribution of these components. Typically, surface areas of the order 500 to 2000 m^2/g have been used.

[0025] In contrast, magnetic particles of the present invention have a surface area less than 50 m^2/g, well below values that would be considered useful for an adsorption/desorption process. In particular, surface areas of iron particles measured by a surface area analyzer, for example BET,

are less than 50 m^2/g and would have been considered too small to allow the adsorption of diagnostic or therapeutic quantities of pharmaceutical compounds in general, and especially of large macromolecules. Additionally, modification of the particle size of the magnetic iron would not have been considered to provide useful alternatives. For example, use of larger particles might preclude intravenous administration, while use of smaller particles might not yield sufficient magnetic susceptibility. Thus, iron particles would not have been considered as potential carriers to deliver therapeutically relevant quantities of biologically active compounds. Indeed, for the same reasons, none of the other instant magnetic particles would have been considered as potential carriers of biologically active compounds.

[0026] Moreover, a useful delivery technology should be inert biologically, and ideally biocompatible or biodegradable. Magnetic particles would, at first, not be considered as the ideal biologically inert system because of toxicity concerns. Thus, for example, the iron particles of the present invention are surprisingly biocompatible. The most likely reason for this is a slow *in vivo* absorption process.

[0027] In view of the concepts in the following description, this invention resides in the novel construction, combination, formulations, kits, arrangement of parts and/or reagents and methods substantially as hereinafter described, and more particularly defined by the appended claims, it being understood that changes in the precise embodiments of the herein disclosed invention are apparent to any person having ordinary skill within the art, and are meant to be included as they come within the scope of the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] Figure 1 is a Scanning Electron Microscope (SEM) picture of metallic iron particles (unprocessed).

[0029] Figure 2 is the size distribution of the iron particles (unprocessed) using a light scattering technique.

[0030] Figure 3 shows the attachment efficiency of rhenium radical onto unprocessed and processed iron particles.

[0031] Figure 4 shows the stability in human plasma of the attached rhenium radical onto two different types of particles.

[0032] Figure 5 is a Scanning Electron Microscope (SEM) picture of metallic iron particles after a high-energy milling treatment.

[0033] Figure 6 shows the stability of Yttrium 90 attached to iron particles in human plasma at 37°C as a function of time.

[0034] Figure 7 shows the stability of rhenium radical attached to iron particles (processed and unprocessed) as a function of time.

DETAILS OF THE INVENTION

[0035] The present invention relates to a single component capable of carrying a biologically active compound in relevant quantities to a specific site. This involves magnetic particles having attached thereto (that is, precipitated, adsorbed, or labeled thereon) one or more biologically active compounds. This combination of magnetic particles and one or more biologically active agents attached thereto is referred to herein as a "magnetically susceptible composition". The magnetic particles have the general properties of having Curie temperatures (Tc) greater than the normal human body temperature (37°C), having high magnetic saturation (> approximately 20 Am^2/kg), and being ferromagnetic or ferimagnetic. Examples of the magnetic particles include iron oxides such as Jacobsite ($MnFe_2O_4$), Trevorite ($NiFe_2O_4$), iron sulfides such as Pyrrhotite (Fe_7S_8), and Greigite (Fe_4S_4), ceramics such as Alnico 5, Alnico 5 DG, Sm_2Co_{17}, $SmCo_5$ and NdFeB, as well as metals and alloys, such as iron (Fe), cobalt (Co), nickel (Ni), awaruite (Ni_3Fe) and wairauite (CoFe). Specifically excluded from the above-descirbed magnetic particles and the instant magnetically susceptible compositions and the embodiments encompassing them are the iron oxides magnetite (Fe_3O_4), hematite (αFe_2O_3), and maghemite (γFe_2O_3). Each of the magnetic particles can have added to its chemical formula specific impurities that may or may not alter the magnetic propreties of the material. Doped ferromagnetic or ferrimagentic materials within the above limits of Curie temperatures and magnetic saturation values are considered to be within the scope of the instant invention. The term "metal particles" as used herein refers to paritcles formed from the metals and alloys listed above, while the term "metallic iron particles" refers to those formed from iron alone. Such particles are available commercially.

[0036] One examplary group of the instant embodiments encompassing the magnetically susceptible compositions occurs when in any of the instant embodiments the magentic particle is selected from the group consisting of iron, nickel, awaruite, wairauite, pyrrhotite, greigite, troilite, yttrium iron garnet, Alnico 5, Alnico 5 DG, Sm_2Co_{17}, $SmCo_5$ and NdFeB particles. Another examplary group of the instant embodiments occurs when the magnetic particle is selected from the group consisting of iron, nickel, awaruite, wairauite, pyrrhotite, greigite, troilite, and yttrium iron garnet particles. Another group of instant embodiments occurs when the particles for use in the instant embodiments are those that have a magnetic saturation value of approximately 100 Am^2/kg or above. Yet another exemplary group of instant embodiments occurs when the particles are ferrimagnetic.

[0037] Such magnetic particles possess superior magnetic susceptibility. A preferred magnetic particle for the various embodiments of the invention described herein is an iron particle. Yet another preferred magnetic particle for the various embodiments of the invention described herein is wairauite (CoFe).

[0038] In particular, the term "iron particles" is meant to include any biocompatible, metallic iron particles, essentially free of carbon. The term "essentially free of carbon" refers to, for example, less than 1% by mass of carbon. Thus, the term refers to, for example, 0.9%, 0.8, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.05% and the like. It also refers to, for example, carbonyl iron powder. The term "metallic iron" is meant to include any magnetically susceptible iron composition having less than about 10-20% by weight iron oxide(s). This also includes a metallic iron phase that is a single solid phase, or a single solid solution, comprised primarily of metallic iron, preferably greater than about 75%. Unlike previous compositions having iron components comprised primarily of iron oxide, the amount of iron oxide in the compositions of the present invention is limited to impurities and thus is present in a small amount. Such iron particles possess superior magnetic susceptibility.

[0039] The "magnetic susceptibility" of the particles is the degree of responsiveness of a sample of the particles to a magnetic field, wherein lack of magnetic susceptibility correlates to an absence of response to a magnetic field. This responsiveness may be affected for example, by the components present in the magnetic composition. The responsiveness lends the particle the ability to be localized in an organism with an externally applied magnetic field. The ability to be localized is affected by the route of administration, by the resulting depth of the particles in the body and/or strength of the magnetic field. The term "biologically active" is meant to include any compound having *in vivo* and ex vivo diagnostic and/or therapeutic properties. The terms "a" and "one" are both meant to be interpreted as "one or more" and "at least one."

[0040] The method used to produce the magnetically susceptible composition uses magentic particles that are essentially ree from organic and inorganic impurities. In the case of the instant iron particles, for particles that are mainly metallic iron are used. For example, the addition of extreme heat or the use of certain chemical processes that result in iron oxidation are not necessary. The iron may be raw, or subjected to processing such as high-energy milling or gas phase treatments. The state and nature of the particle surface is not critical to the attachment efficiency. However, the particle surface may be optimized, for example, to enhance attachment, bioavailability and targeting efficiency, and/or to increase surface area without change to the overall particle size. Because the "carrier" is a magnetic particle rather than an additional component, the particles display an extremely high level of magnetic susceptibility, while at the same time exhibiting unexpected but excellent attachment efficiency.

[0041] If desired, the magnetic particles can be processed to change their shape, size, surface area, and surface chemistry before biologically active compounds are attached to them. Many different processes can be used to increase and to optimize either the magnetic susceptibility of the particles or the resulting amount of the biologically active compounds that can be attached to the particles. For example, raw particles such as iron can undergo gas phase treatment or activation, milling, thermal activation, chemical vapor deposition of functional groups or any of a variety of

other techniques apparent to any person skilled in the art. (See, for example, Reynoldson, R.W. "The use of fluidized bed reactors for chemical vapor deposition, thermochemical diffusion treatment on ferrous and non-ferrous alloys" Heat Treatment of Metals, 28(1), 15-20, (2001); ;Ucisik, A.H, Zeytin, S., Bindal, C., "Boride coating on iron based alloys", J. Australasian Ceramic Soc., 37, (2001); H. Isaki, M. Yoshino, M Kurotobi, M Uchida, H Shimizu, "Treatment of Surface of Ferrous Material" Japanese Patent 08320100, 1996; and Pantelis, D, Pantazopoulous, G., "Large scale pulsed laser surface treatment of a lamellar graphite cast iron", Surface Modification Technologies VIII. Proceedings, 8th International Conference, Nice, France, 26-28 Sept. 1994, eds. T.S. Sudarshan, M. Jeandin, J.J. Stiglich, W. Reitz. Publ: London SW1Y 5DB, UK The Institute of Materials, 297-309, (1995).

[0042] For example, but without limitation, the magnetic particles may be milled, as described below. This milling step could result in particles with higher magnetic moment because of the particles' deformation during the process. The deformation leads to elongation of the particles, such that, when in the presence of a magnetic field, magnetic poles are established on the most spatially distant ends of the particles. The spatial separation could lead to a quantitatively higher magnetic moment induced in the particle, giving it superior targeting capabilities compared to an equivalent mass of perfectly spherical magnetic particles. It could also lead to improvements in the particle's attachment efficiency with the biologically active material to be carried.

[0043] Thus, for example, the magnetic particles used in the various aspects of the instant magnetically susceptible compositions have a size of less than about 1 mm, preferably between 0.1 and 20 microns, and most preferably between 0.2 and 5 microns. Typically, the magnetic material such as iron used for making the particles is essentially chemically pure, free of carbon, with higher than about 75% magnetic material, more preferably higher than about 85% magnetic material, and most preferably higher than about 95%. Regarding the embodiments of the instant magnetically susceptible compositions with iron particles, the magnetic material used for making the particles also typically contains less than about 20% iron oxides, more preferably less than about 10%, and most preferably less than about 5%.

[0044] A number of considerations are involved in determining the size of carrier particles to be used for any specific diagnostic and/or therapeutic situation. The choice of particle size is determined in part by technological constraints inherent in producing the magnetic particles such as iron under 0.2 micron in size. On the other hand, relatively large particle sizes can cause desirable or undesirable embolization of blood vessels during injection either mechanically or by facilitating clot formation by physiological mechanisms. The dispersion may coagulate, which makes injections more difficult, and the rate at which biologically active compounds desorb from the magnetic particles in the targeted pathological zones may decrease.

[0045] In view of the above considerations, the size of the magnetic particles should be less than 50 microns, preferably less than 30 microns, and more preferably less than 10 microns. Generally,

the particles have an average size in the range of 0.2 to 5 microns with 99% of the particles below 10 microns. These size ranges are exemplary of the embodiments of the instant invention that encompass an iron particle. Any person having ordinary skill in the art can readily determine the size range of particles to be used for any given application and route of administration. The size of the resulting particles can be controlled by the size of the magnetic particles used, or by typical size selection processes that may be employed before or after the biologically active compounds have been attached.

[0046] In the case of iron, the particles may be raw iron particles or processed iron particles. As seen in the examples given below, use of either raw or processed iron particles can affect the adsorption, precipitation, or labeling of biologically active compounds onto the magnetic particles, as well as affecting the stability as a function of time and the magnetic susceptibility. Depending on the characteristics desired, processes may be used singly or in combination. Processes that may be employed include milling, chemical vapor deposition, or gas phase treatment. Other suitable processes are apparent to those having skill within the art. These processes are useful in manipulating the other magnetic particles, as well.

[0047] The high-energy milling process consists of combining the magnetic powder with a liquid, for example ethanol, in a canister containing grinding balls. The liquid serves as a lubricant during the milling process and also inhibits the oxidation of the powder, an especially important consideration when fabricating the instant magnetic particles. The canisters are then placed in a laboratory planetary mill of the type characteristically used in metallurgy (*i.e.*, mill made by Fritsch, Germany). Other types of mills producing similar results may also be employed. The mill is run for an appropriate time (generally between 1 and 10 hours) at speeds, for example, between 100 and 1000 rpm. At the end of the cycle, the magnetic particles are collected. The particles may be re-suspended and homogenized if desired. The magnetic particles are dried by any suitable technique, allowing for the protection of the particles against oxidation, or in the case of yttrium iron garnet, for example, against further oxidation. This process results in elongation of particles, rendering them more magnetically susceptible due to increased pole separation, and larger surface area per mass of magnetic substance.

[0048] Another process includes subjecting the particles to a gas phase treatment. For example, iron particles may be placed in a quartz container within an oven. Hydrogen may be used to replace air in the oven and the temperature is then raised for example, to about 300°C. The iron particles are left in this environment for about 2 hours. At the end of the cycle, the temperature is lowered and hydrogen is replaced by nitrogen. Once the iron particles' temperature has been returned to room temperature, they are collected and packaged. This process results in an increase in the roughness of the magnetic particle surface, leading to enhanced attachment of the biologically active compound.

[0049] The biologically active compound may be introduced to the raw magentic particles or to particles that have been processed, if desired to form the instant magnetically susceptible compositions Various methods of attachment (that is, labeling, adsorbing and/or precipitating) biologically active compounds are known in the art. (See, for example, Harrison, RG, Todd, PW, Rudge, SR and Petrides, DP, Bioseparation Science and Engineering, Chapters 8,9, and 10, Oxford University Press, New York, 2003.) The specific parameters used in these processes will depend upon the character and quality of the surface of the magnetic particles as well as that of the biologically active compound(s), and the properties of the solutions employed.

[0050] Further depending on the characteristics of the biologically active compounds to be attached to the magnetic particles (for example, molecular weight, chemical structure, redox properties, and solubility), any person having ordinary skill in the art may easily identify an appropriate method for introduction of the desired biologically active compound(s). (See, for example, Harrison, RG, Todd, PW, Rudge, SR and Petrides, DP, Bioseparation Science and Engineering, Chapters 8,9, and 10, Oxford University Press, New York, 2003). For instance, it is known that the reduction of perrhenate leads to insoluble rhenium oxides; thus, a redox reaction would be a good choice for labeling magnetic particles with rhenium oxides.

[0051] As another example, the magnetic particles may be incubated with the biologically active compound in a medium, for example, water, buffer, or solvent to form the instant magnetically susceptible compositions. Preferably, the medium should not include compounds that are likely to solubilize the magnetic particles. Initially, the amount of incubation time may be determined in the feasible and reasonable range of about 5 to about 90 minutes, and preferably in the range of about 15 to about 60 minutes. The incubation temperature may be determined in accordance with the stability of the desired biologically active compounds. The incubation times and temperatures may be adjusted to obtain the highest efficiency.

[0052] Additional methods include the addition of solvent, such as ethanol, addition of salt or change of pH so as to induce precipitation, evaporation, or reduction of volume. Another possible method may include lowering the temperature of the solution in which the biologically active compound is present so as to induce precipitation or crystallization of the compound. Any person having ordinary skill in the art would be familiar with the appropriate methods involved in attachement and would be able to adjust the methods accordingly without undue experimentation.

[0053] Chemicals may be introduced to the process, for example, in order to alter the solubility of the biologically active compounds, to induce precipitation (for instance, a redox reaction), or to facilitate attachment onto the particles (for example, pH modification, or adjustment of the hydrophilicity-lipophilicity balance of the solution). These chemicals may be included in the solution containing the biologically active compound or introduced after the magnetic particles have been added. Time, temperature, and conditions of the incubation reaction, as well as use of an

additional excipients or chemical substances, may be adapted to the properties and characteristics of the biologically active compound(s) to be attached to the magnetic particles.

[0054] The magnetic particles may be optionally washed, dried, recovered, sterilized and/or filtered. Routine methods of packaging and storing may be employed. For example, the raw or processed dried particles such as iron may be packaged in appropriate container closure system, for example, one enabling unit dosage forms. Packaging under nitrogen, argon or other inert gas is preferred to limit the oxidation of particles such as iron. The iron particles must be stored so as to prevent oxidation. Although the particles may be stored "wet," the liquid should not be aqueous. For example, ethanol or DMSO may be employed. (See, for example, Kibbe, AH, Handbook of Pharmaceutical Excipients, American Pharmaceutical Association, Washington, DC, 2000.) The particles may be sterilized by any appropriate means, keeping in mind that methods relying on moist heat, such as by autoclave, may tend to lead to undersirable oxidation or futher oxidation of the particles.

[0055] Because it is convenient to prepare and market the magnetic particles in a dry form, the excipients may be prepared in dry form, and one or more dry excipients are packaged together with a unit dose of the carrier particles. A wide variety of excipients may be used, for example, to enhance precipitation or release of the biologically active compound. The type and amount of appropriate dry excipients can readily be determined by any person having ordinary skill in the art. For instance, the excipients can be selected from a viscosity agent or a tonicifier, or both. Viscosity agents are, for example, biodegradable polymers such as carboxymethylcellulose, PVP, polyethylene glycol (PEG), and the like. Tonicifiers include sodium chloride, mannitol, dextrose, lactose, and other agents used to impart the same osmolarity to the reconstituted solution. Most preferably, the package or kit containing both the dry excipients and dry magentic particles such as iron is formulated to be mixed with the liquid contents of a vial containing a unit dose of the biologically active compound. Liquid agents could be used as excipients just prior to use of the particles. Such liquid agents could be soybean oil, rapeseed oil, or an aqeous based polymer solution composed of the polymers as listed above. Also liquid solutions could be a tonicifier, such as Ringer's solution, 5% dextrose solution, physioligical saline. As before a combination of liquid excipients and tonicifiers can be used. (See, for example, Kibbe, AH, Handbook of Pharmaceutical Excipients, American Pharmaceutical Association, Washington, DC, 2000).

[0056] Upon mixture of the liquid containing the biologically active compound with the contents of the kit including the dry components (*i.e.*, the dry iron particles and dry excipients), the biologically active compound attaches to the magnetic particles according to a protocol developed for each compound, thus forming a magnetically controllable composition containing a diagnostic and/or therapeutic amount of a biologically active compound attached to the magnetic particles and being suitable for *ex vivo* or *in vivo* therapeutic and/or diagnostic as well as ex vivo diagnostic use. Any suitable sterilization technique may be employed. For example, iron particles may be

sterilized using gamma or electron irradiation or dry heat and the aqueous solution of excipients may be sterilized by autoclave.

[0057] The resulting particles having attached thereon one or more biologically active compounds ("magnetically susceptbile compositions") may be used alone or incorporated into a delivery system. Suitable delivery systems will be apparent to any person possessing ordinary skill in the art. Without limitation, examples of useful delivery systems include matrices, capsules, slabs, microspheres, and liposomes. Conventional excipients may be incorporated into any of the formulations.

[0058] A diagnostic and/or therapeutic amount of a biologically active compound attached to the magnetic particles will be determined by any person having ordinary skill in the art as that amount necessary to effect diagnosis and/or treatment of a particular disease or condition, taking into account a variety of factors such as the patient's weight, age, and general health, and the nature and severity of the disease. Typically one dose of the magnetically susceptible composition will deliver a unit dose of the biolgically active compound.

[0059] Magnetic particles provide superior delivery of biologically active compounds to specific sites in the body under the influence of a magnetic field as a result of the combination of the high magnetic susceptibility of the microparticles and the biologically active compound carrying capability of the magnetic particle. For instance, the particles have a significantly higher magnetic susceptibility due to the use of metallic magnetic iron instead of iron oxides. Magnetic susceptibility of iron oxides, for similar size and shape particles, can be as much as ten times lower than pure metallic magnetic iron particles. Consequently, magnetic iron particles can be targeted more efficiently and at greater depths within the body than iron oxide based particles, with less sophisticated magnet technology, and if desired, without requiring a carrier component such as a polymer matrix.

[0060] In addition to *in vivo* applications, the instant magnetically susceptible compositions of the invention may also be used *ex vivo*. For example, and without limitation, biological material may be extracted from a patient and dispersed in a slurry. The magnetic particles having attached thereon a biologically active compound may be present in the test container either before or after the biological material is dispersed. Application of a magnetic field separates the biological material related to the disease due to the material's "recognition" of the biologically active compound attached to the metallic magnetic particle. For example, this recognition may be via binding.

[0061] Generally, any useful diagnostic and/or therapeutic compound may be attached to the magnetic particles such as iron for guided delivery to a target site. The term "biologically active" also includes compounds used for diagnostic purposes and having no apparent physiological, therapeutic effect. Bifunctional compounds having both diagnostic and therapeutic properties are also contemplated. Biologically active compounds that can be attached to the magnetic particles

are, for example, but not limited to muscarinic receptor agonists and antagonists; anticholinesterase agents; catelcholamines, sympathomimetic drugs, and andrenergic receptor antagonists; serotonin receptor agonists and antagonists; local and general anesthetics; anti-migraine agents such as ergotamine, caffeine, sumatriptan and the like; anti-epileptic agents; agents for the treatment of central nervous system degenerative disorders; opiod analgesics and antagonists; anti-inflammatory agents, including anti-asthmatic drugs; histamine and bradykinin antagonists, lipid-derived autocoids; nonsteroidal antiinflamatory agents and anti-gout agents; anti-diuretics such as vassopressin peptides; inhibitors of the renin-angiotensin system such as angiotensin converting enzyme inhibitors; agents used in the treatment of myocardial ischemia, such as organic nitrates, Ca^{2+} channel antagnonists, beta-adrenergic receptor antagonists, and antiplatelet/antithrombotic agents; anti-hypertensive agents such as diureitcs, vasodilators, Ca^{2+} channel antagnonists, beta-adrenergic receptor antagonists; cardiac glycosides such digoxin, phosphdiesterase inhibitors; antiarrhythmic agents; anti-hyperlipoprotenimia agents; agents for the control of gastric acidity and treatment of peptic ulcers; agents affecting gastrointestinal water flux and motility; agents that cause contraction or relaxation of the uterus; anti-protozoal agents; anthelmintic agents; antimicrobial agents such as sulfonamides, quinolines, trimethoprim-sulfamethoxazole; beta-lactam antibiotics; aminoglycosides; tetracyclines; erythromycin and its derivatives; chloramphenicol, agents used in the chemotherapy of tuberculosis; *Mycobacterium avium* complex disease, and leprosy; anti-fungal agents; and anti-viral agents; anti-neoplastic agents such as alkylating agents, antimetabolites; natural products such as the vinca alkaloids, antibiotics (e.g., doxorubicin, bleomycin and the like); enzymes (e.g. L-asparaginase), biological response modifiers (such as interferon-alpha); platinum coordination compounds, anthracenedione and other miscellanous agents; as well as hormones and antagonists (such as the estrogens, progestins, and the adrenocorticosteriods) and antibodies; immunomdulators including both immunosuppressive agents as well as immunostimulants; hematopoietic growth factors, anticoagulant, thrombolytic and antiplatelet agents; thyroid hormone, anti-thyroid agents, androgen receptor antagonists; adrenocortical steroids, insulin, oral hypoglycemic agents, agents affecting calcification and bone turnover as well as other therapeutic and diagnostic hormones, vitamins, minerals blood products biological response modifiers, diagnostic imaging agents, as well as paramagnetic and radioactive particles Other biologically active substances may include, but are not limited to monoclonal or other antibodies, natural or synthetic genetic material and prodrugs.

[0062] As used herein, the term "genetic material" refers generally to nucleotides and polynucleotides, including nucleic acids, RNA and DNA of either natural or synthetic origin, including recombinant, sense and antisense RNA and DNA. Types of genetic material may include, for example, genes carried on expression vectors, such as plasmids, phagemids, cosmids, yeast artificial chromosomes, and defective (helper) viruses, antisense nucleic acids, both single and

double stranded RNA and DNA and analogs thereof. Also included are proteins, peptides and other molecules formed by the expression of genetic material.

[0063] The biologically active compounds for the instant compositions may also be radioisotopes. Such radioisotopes are chemical compounds or elements that emit alpha, beta or gamma radiation and that are useful for diagnostic and/or therapeutic purposes. One factor used in selecting an appropriate radioisotope is that the half-life be long enough so that it is still detectable or therapeutic at the time of maximum uptake by the target, but short enough so that deleterious radiation with respect to the host is minimized. Selection of an appropriate radioisotope would be readily apparent to one having ordinary skill in the art. Generally, alpha and beta radiation are considered useful for local therapy. Examples of useful compounds include, but are not limited to ^{32}P, ^{186}Re, ^{188}Re, ^{123}I, ^{125}I, ^{131}I, ^{90}Y, ^{166}Ho, ^{153}Sm, ^{142}Pr, ^{143}Pr, ^{149}Tb, ^{161}Tb, ^{111}In, ^{77}Br, ^{212}Bi, ^{213}Bi, ^{223}Ra, ^{210}Po, ^{195}Pt, ^{195m}Pt, ^{255}Fm, ^{165}Dy, ^{109}Pd, ^{121}Sn, ^{127}Te, ^{103}Pd, ^{177}Lu, and ^{211}At. The radioisotope generally exists as a radical within a salt, although exceptions such as iodine and radium exist wherein the radical is not in ionic form. The useful diagnostic and therapeutic radioisotopes may be used alone or in combination.

[0064] For *in vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay that is detectable for a given type of instrument. Generally, gamma radiation is preferred. Still another important factor in selecting a radioisotope is that the half-life be long enough so that it is still detectable at the time of maximum uptake by the target, but short enough so that deleterious radiation with respect to the host is minimized. Selection of an appropriate radioisotope would be readily apparent to one having average skill in the art. Radioisotopes that may be employed include, but are not limited to ^{99m}Tc, ^{142}Pr, ^{161}Tb, ^{186}Re, and ^{188}Re. Additionally, typical examples of other diagnostically useful compounds are metallic ions including, but not limited to ^{111}In, ^{97}Ru, ^{67}Ga, ^{68}Ga, ^{72}As, ^{89}Zr, and ^{201}TI. Furthermore, paramagnetic elements that are particularly useful in magnetic resonance imaging and electron spin resonance techniques include, but are not limited to ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, and ^{56}Fe.

[0065] The methods of use for the instant magnetically susceptible compositions include methods for localized *in vivo* or *ex vivo* diagnosis and/or therapeutic treatment providing a magnetic particle having attached thereto one or more biologically active compounds selected for efficacy in diagnosing and/or treating the disease, and magnetically guiding the particle to a desired location in the body of a patient. Such magnetically susceptible compositions containing said magnetic particles may be introduced enterally or parenterally, by any conventional route of administration.

[0066] For example, the particles attached to the biologicaly active compound(s) may be injected by inserting delivery means such as a catheter or needle into an artery within a short distance from a body site to be treated and at a branch or branches, preferably the most immediate, to a network of arteries carrying blood to the site. The particles are injected through the delivery means into the

blood vessel. Just prior to injection, a magnetic field is established exterior to the body and adjacent to the target site, and having sufficient field strength to guide a substantial quantity of the injected particles to, and retain the substantial quantity of the particles at the site. Preferably, the magnetic field is of sufficient strength to draw the particles into the soft tissue at the site adjacent to the network of vessels, thus avoiding substantial embolization of any of the vessels by the carrier particles, should embolization be undesirable for the particular treatment/diagnosis. One example of such a magnet would be to use either a DC electromagnet or permanent magnent of sufficient size and strength to produce 100 gauss of magnetic flux at the target site of the magnetically susceptible compositions. For example, the magnets discussed in Mitchiner *et al.*, U. S. Pat. No. 6,488,615, issued Dec. 3, 2002, are suitable for use with the instant invention.

[0067] In the case where the biologically active compound(s) includes a diagnostic imaging agent, the imaging is performed while the particles attached to the biogically active compound(s)are captured at the target site, and in some cases before and/or after. Imaging modalities and methods are well-known to any person having ordinary skill in the art. Once the magnetic field is removed, the particles remain at the site for a period of time and then slowly biodegrade. The magnetic field may be applied one or more times in order to guide the particles to one or more desired sites.

[0068] In view of the foregoing disclosure, the following examples are provided to more fully illustrate various embodiments of the invention. The examples are not intended to be limiting in any way, but merely representative of the broader concepts that are disclosed herein.

Example 1 – Method for Milling Iron Particles

[0069] 30 g of metallic iron (Alfa Aesar, Massachusetts; Strem Chemicals, Massachusetts) was processed by placement in a milling canister with 30 ml of ethanol and milling balls. The milling was performed three times for twenty-minute cycles at approximately 200 rpm. The particles were separated from the milling balls, washed with ethanol, and dried under vacuum. The microparticles were sieved in order to separate particles of a particular size range and also to remove undesired solid particulates. The particles were dried in a vacuum and packaged under nitrogen gas. The resulting particles are illustrated in Figure 5.

Example 2 – Method for Gas Phase Treatment of Iron Particles

[0070] Iron particles can be treated with different gases and plasma to increase the surface roughness in order to enhance the binding affinity of biologically active compounds. For example, 4 g of iron particles in a quartz container are placed in an oven. The particles are heated to 300°C in a flow of ultrahigh-purity ammonium (NH_3) at 25 mL/min. After treatment for 3 h, the samples are cooled to room temperature and NH_3 is replaced with N_2.

Example 3 – Labeling of Iron Particles by the Reduction of Perrhenate

[0071] Iron particles (mean diameter of 1.09 μm with 99% of the population smaller than 2.34 μm; see Figures 1 and 2) were used to carry out a protocol based upon the reduction of perrhenate with tin chloride. A ^{188}Re solution obtained from a Tungsten/Rhenium generator (Department of

Energy, Oak Ridge National Laboratories) was concentrated to 4.00 mCi Re-188 in 1.0 mL of saline. A tin chloride solution (20 mg/mL $SnCl_2$) was prepared by dissolving 20.1 mg $SnCl_2$ in 0.5 mL of 0.2 N HCl, and then by adding 0.5 mL 0.9% saline; the mixture was vortexed until dissolution. Raw iron particles (20 mg, Alfa Aesar, 1-3 μm) were weighed out in three tubes each. For each sample, we added:

- 100μL tin solution and mix,
- 125 μL saline and mix,
- 100μL of Re-188 solution and vortex 30 s (leading to ~.4mCi/tube per sample),

and the tubes were incubated for 1 hr, 70°C at 1400 rpm on a thermomixer. After incubation, the samples were washed: 675μL PBS pH 7.4 was added to each tube and the activity was measured in the Rad Cal dose calibrator; supernatants were removed by isolating the particles with a magnet or by centrifugation, then 1 mL PBS pH 7.4 was added and the tubes were vortexed for 30 sec. The ratio of the activity remaining on the microparticles after the two washing steps divided by the initial activity gives the percentage of activity effectively attached to the microparticles (noted labeling efficiency in Figure 3 as "(1-3) Fe").

Example 4 – Stability Testing

[0072] The labeling stability of the samples from Example 3 was tested in human plasma. Iron particles with rhenium were placed in human plasma in a shaking water bath at 37°C for 48 hours. Measurement of the activity remaining on the iron particles was measured as a function of time. Labeling stabilities are shown in Figure 4 as "(1-3) Fe". Temperatures between 25°C and 100°C and reaction times between 5 minutes and 2 hours are acceptable. Typically, the higher temperatures and longer reaction times are preferred. The conditions used in this experiment were chosen based on practical considerations for the perrhenate, and may be adjusted when using another compound to be adsorbed onto the particles.

[0073] Results show that the labeling (attachment) efficiency and stability of the iron particles was very good. Stability of the labeling or adsorption is important when a radioisotope is to be delivered to the specific site in the body. One would like to minimize the activity present in the systemic circulation in order to minimize side effects by keeping the activity bound or adsorbed onto the particles over time.

Example 5 – Labeling and Stability for Milled Particles

[0074] The same experiment was repeated but using milled iron particles (less spherical particles used in the previous Example with a mean of 0.97 μm and with 99% of the population smaller than 2.18 μm). Results are also shown in Figure 3 and 4 (as "milled Fe"). Comparison of the labeling efficiencies and stabilities in human plasma surprisingly indicated that milled iron particles had a slightly better labeling efficiency and stability than raw iron particles.

Example 6 – Iron Particles Labeled with Yttrium 90

[0075] Iron particles can also be labeled with Yttrium 90. In the case of Yttrium 90, there is no addition of tin chloride to oxidize the biologically active compound. The labeling is obtained by direct adsorption of the $^{90}Y^{+3}$ onto the iron particles. The adsorption was performed as follows: 20 mg of iron particles in 1.5 mL were placed in a screw cap tube, and 100 μL of an ammonium acetate buffer with ~0.5 mCi $^{90}YCl_3$ was added (NEN Perkin-Elmer). The tubes were incubated at 37 °C for 30 min on a thermomixer at 1400 rpm. After the incubation, PBS buffer pH 7.4 was added to bring the volume to 1 mL (wash-1). Activity was measured. Then the supernatant was removed. 1 mL of fresh PBS buffer was added into the tube. The activity of the whole tube was measured again. Then the supernatant was removed (wash-2). 1.5 mL of human plasma was added into the tube for stability measurement. The activity of the whole tube was measured again. Labeling efficiency was determined as the ratio of the final activity on the particles to the total activity used. The stability is the percent of the labeled radioactivity that remains on the particles in human plasma as a function of time. Results (Figure 6) showed about 60% of Yttrium 90 was bound onto iron particles with a stability of greater than 90% in human plasma after 70 hours, indicating that Yttrium 90 is strongly adsorbed onto iron particles.

Example 7 – Increased Reaction Temperature

[0076] Experiments described in Example 3 were repeated but the temperature of the reaction was increased from 70°C to 99°C. The stability of the binding was investigated in human plasma at 37°C over 24 hours as described in Example 4. Figure 7 shows the results for the raw and milled iron particles. Both iron particles have excellent binding stability.

Example 8 – Binding of Tumor Necrosis Factor α (TNFα)

[0077] Small molecules or large macromolecules can be adsorbed onto iron particles. TNFα is a 17 kDa cytokine that can induce hemorrhagic necrosis of cancerous cells and tumors. Carrier-free TNFα was suspended in water at neutral pH. 200 μL of TNFα solution was mixed with 1 mg of iron particles. The iron particles were incubated with the TNFα for 1 hour at room temperature on a plate shaker. After incubation, the particles were separated using a magnet, and the supernatants were removed. The particles were washed twice with 200 μL PBS at pH 7.4 for 1 mg of iron, and the wash supernatants were saved for analysis. The desorption of TNFα from the particles was tested by adding 200 μL plasma to the particles and incubating the plate at 37°C with shaking. The plasma was changed at 30 min, 1 hr, 2 hr, and 4 hrs to give a semi-dynamic desorption curve. The concentrations of TNFα in samples were measured using ELISA plate.

[0078] Results showed that more than 60% (1.2 μg) of TNFα were bound onto the iron particles when 2 μg TNFα/mg Fe ratio was used. At this binding level, only 7% of TNFα desorbed from the particles after 4 hours in an aqueous based viscous re-suspension media used for parenteral administration (10% mannitol and 0.5% carboxymethoxycellulose in water for injection) while 60%

of TNFα was released after four hours in human plasma. These results indicate that TNFα could be bound onto the iron particle and TNFα/Fe complex was stable in aqueous based viscous re-suspension media and that TNFα was effectively released from the microparticles in human plasma. Indeed, while a radioisotope should not be released from the iron particles, in the case of a chemical compounds, the compound should be released from the particles.

Example 9 - Magnetic Capture Test

[0079] An *in vitro* magnetic capture test has been designed to assess the percentage of the test material captured by the magnetic field. The test material is in suspension in a 10% w PEG (polyethylene glycol) water solution. Using a peristaltic pump, the test material suspension is pumped into tubing positioned in front of a 39H permanent magnet (Magnet Sales & Mfrg., Culver City, CA; Dexter Corp., Richardson, TX) capable of producing 0-0.7 tesla (0-7,000 gauss). The inner diameter of the tube is between 0.5 and 10 mm. The magnet can be moved away from the tube in order to change the flux of the magnetic field (gauss; tesla) applied on the test material in suspension. With the magnet positioned 4 cm away from the tubing and a magnetic field of about 0.1 tesla (1 kgauss), 99% of the test material was captured by the magnetic field.

What Is Claimed

1) A magnetically susceptible composition comprising a magnetic particle that has attached thereon a biologically active compound.

2) The composition of claim 1 wherein the magnetic particle is selected from the group consisting of raw, synthetic, processed, activated, and combinations thereof.

3) The composition of claim 2 wherein the magnetic particles has undergone a process selected from the group consisting of high-energy milling, gas phase treatment, and combinations thereof.

4) The composition of claim 2 wherein the magnetic particle has undergone activation of the type selected from the group consisting of thermal activation, chemical vapor deposition of functional groups, and combinations thereof.

5) The composition of claim 1 wherein the biologically active compound is attached by a method selected from the group consisting of labeling, adsorption, precipitation, and combinations thereof.

6) The composition of claims 1 through 5, wherein the magnetic particle is an iron particle.

7) The composition of claim 6 wherein the percentage of iron in the particle is greater than about 75%.

8) The composition of claim 6 wherein the percentage of iron in the particle is greater than about 85%.

9) The composition of claim 6 wherein the percentage of iron is in the particle is greater than about 95%.

10) The composition of claim 6 wherein the average particle size is less than about 1 mm.

11) The composition of claim 6 wherein the average particle size is from about 0.1 μm to about 20 μm.

12) The composition of claim 6 wherein the average particle size is from about 0.2 μm to about 5 μm.

13) The composition of claim 6 wherein the biologically active compound is an antineoplastic agent.

14) The composition of claim 6 wherein the biologically active compound is doxorubicin.

15) The composition of claim 6 wherein the biologically active compound is TNFα.

16) The composition of claim 6 wherein the biologically active compound is ^{188}Re.

17) The composition of claim 6 wherein the biologically active compound is ^{90}Y.

18) Use of a magnetically susceptible composition for the manufacture of a medicament, wherein said composition comprises a magnetic particle having attached thereon a biologically active compound.

19) The use of claim 18 wherein the magnetic particle has a therapeutically effective amount of a biologically active compound attached thereon.

20) The use of claim 18 wherein the magentic particle has a diagnostically effective amount of a biologically active compound attached thereon.

21) The use of claims 18 through 20 wherein the magnetic particle is an iron particle.

22) The use of claim 19 whererin the biologically active compound is an antineoplastic agent.

23) The use of claim 19 wherein the biologically active compound is doxorubicin.

24) The use of claim 19 wherein the biologically active compound is chosen from the group consisting of TNFα, ^{188}Re, and ^{90}Y.

25) Use of a magnetically susceptible composition for the treatment of disease in a patient in need thereof, wherein said composition comprises a magnetic particle having attached thereon a biologically active compound.

26) The use of claim 25, wherein the magnetic particle is an iron particle.

27) Use of a magnetically susceptible composition for *in vivo* diagnostic imaging in a patient comprising:

a) Establishing a magnetic field exterior to the body of said patient adjacent to the site to be imaged;

b) administering to said patient a magnetically responsive composition comprising a magnetic particle having attached thereon a biologically active compound;

c) producing an image based upon magnetic detection of said magnetically responsive composition; and

d) analyzing said image to provide a diagnosis.

28) The use of claim 27, wherein the magnetic particle is an iron particle.

29) Use of a magnetically susceptible composition for *ex vivo* diagnostic imaging comprising:

a) providing a combination of a biological material and a magnetically susceptible composition that comprises a magnetic particle having attached thereon a biologically active compound;

b) applying a magnetic field to said combination; and

c) analyzing said biological material to provide a diagnosis.

30) The use of claim 29, wherein the magnetic particle is an iron particle.

31) Use of the composition of any of claims 1-12 in the diagnosis of disease in a patient.

32) The use of the composition of any of claims 1-12 in the *in vivo* diagnosis of disease in a patient.

33) The use of the composition of any of claims 1-12 in the *ex vivo* diagnosis of disease in a patient.

34) Use of the composition of any of claims 1-17 in the treatment of disease in a patient in need thereof.

35) A kit for administering a biologically active substance comprising:

a) a first receptacle comprising a unit dose of magnetic particles; and

b) a second receptacle comprising a solution comprising one or more biologically active compounds.

36) The kit of claim 35 wherein the first receptacle further comprises dry excipients.

37) The kit of claim 35 wherein the magnetic particles are metallic iron particles.

38) A method for local regional therapy comprising:

a) intra-arterial injection of a magnetically responsive composition comprising a magnetic particle having attached thereon a biologically active compound; and

b) establishment of an external magnetic field adjacent to a desired target region.

39) A method of claim 38, wherein the magnetic particle is a magnetic iron particle.

40) A method for increasing the concentration of a biologically active compound at an *in vivo* site comprising:

a) injecting into a patient a magnetically responsive composition comprising a magnetic particle having attached thereon a biologically active compound; and

b) establishing an external magnetic field adjacent to the *in vivo* site where said increased concentration is desired.

41) The method of claim 40, wherein the magnetic particle is a magnetic iron particle.

42) A process for producing a magnetically susceptible composition comprising attaching a biologically active compound onto a magnetic particle.

43) The process of claim 42 wherein the attaching method is selected from the group consisting of labeling, adsorption, precipitation, and combinations thereof.

44) The process of claim 42 wherein said magnetic particle is selected from the group consisting of raw, synthetic, processed, activated, and combinations thereof.

45) The process of claim 44 wherein the magnetic particle undergoes a process selected from the group consisting of high-energy milling, gas phase treatment, and combinations thereof.

46) The process of claim 44 wherein the magnetic particle undergoes activation selected from the group consisting of thermal activation, chemical vapor deposition of functional groups, and combinations thereof.

47) The process of claim 42 through 46, wherein the magnetic particle is a metallic iron particle.

48) The process of claim 47 wherein the percentage of iron in the particle is greater than about 75%.

49) The process of claim 47 wherein the percentage of iron in the particle is greater than about 85%.

50) The process of claim 47 wherein the percentage of iron in the particle is greater than about 95%.

51) The process of claim 47 wherein the average particle size is les than about 1 mm.

52) The process of claim 47 wherein the average particle size is from about 0.1 μm to about 20 μm.

53) The process of claim 47 wherein the average particle size is from about 0.2 μm to about 5 μm.

54) The process of claim 47 wherein the biologically active compound is an antineoplastic agent

55) The process of claim 47 wherein the biologically active compound is doxorubicin.

56) The process of claim 47 wherein the biologically active compound is TNFα.

57) The process of claim 47 wherein the biologically active compound is ^{188}Re.

58) The process of claim 47 wherein the biologically active compound is ^{90}Y.

59) A method for local regional therapy comprising:

a) intravenous injection of a magnetically responsive composition comprising a magnetic particle having attached thereon a biologically active compound; and

b) establishment of an external magnetic field adjacent to a desired target region.

60) A method of claim 59, wherein the magnetic particle is an iron particle.

61) A composition made by the process comprising attaching a biologically active compound onto an iron particle.

62) A magnetically susceptible composition comprising an iron particle having attached thereon a biologically active compound, whereby the composition is produced by a process comprising high-energy milling of said iron particle.

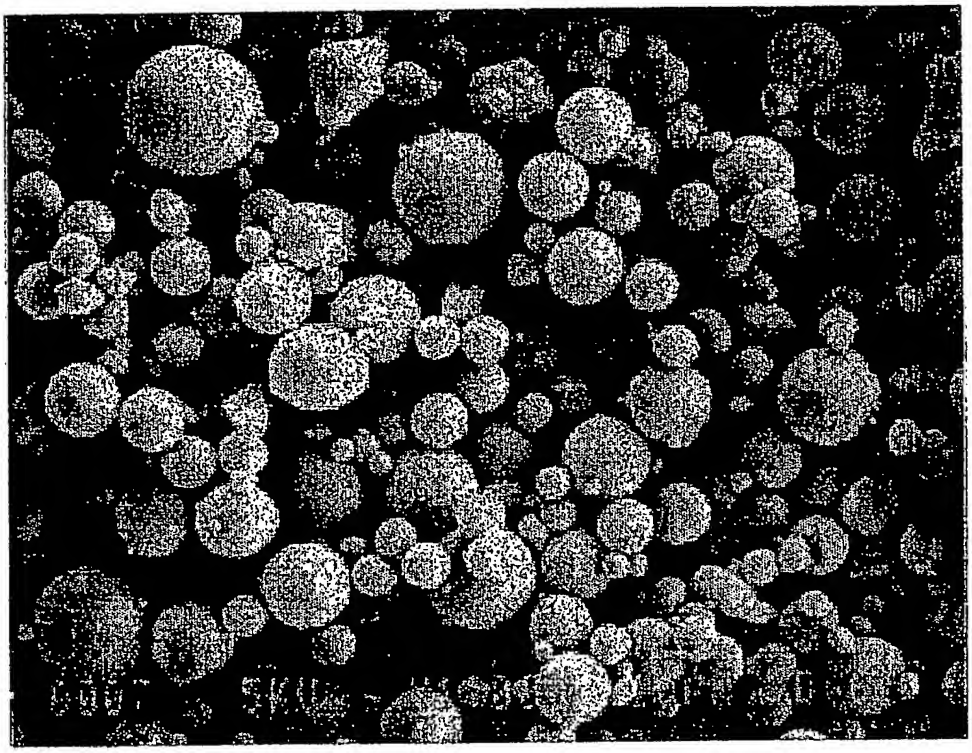

FIG. 1

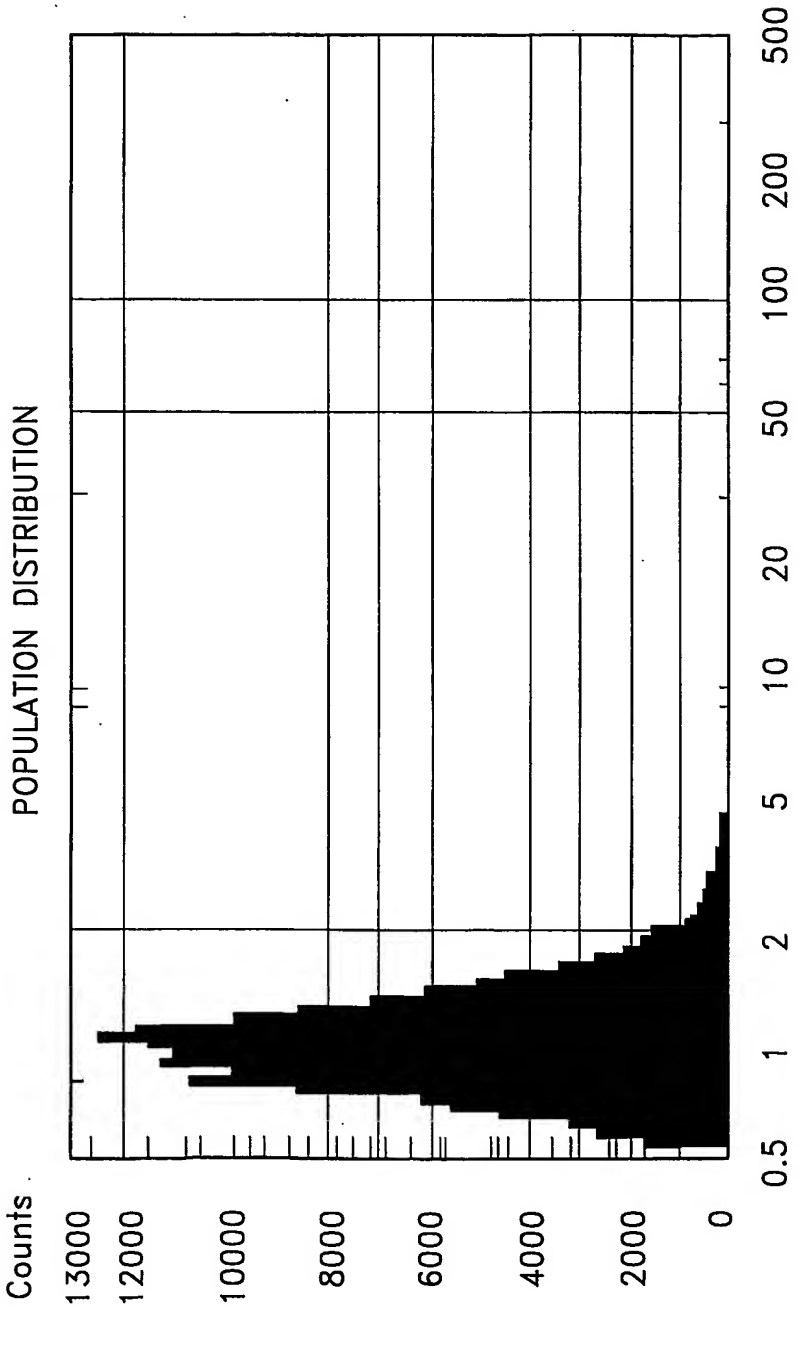
POPULATION DISTRIBUTION
Counts
13000
12000
10000
8000
6000
4000
2000
0
0.5
1
2
5
10
20
50
100
200
500
Diam. (um)->
RAWIRON.1

FIG. 2

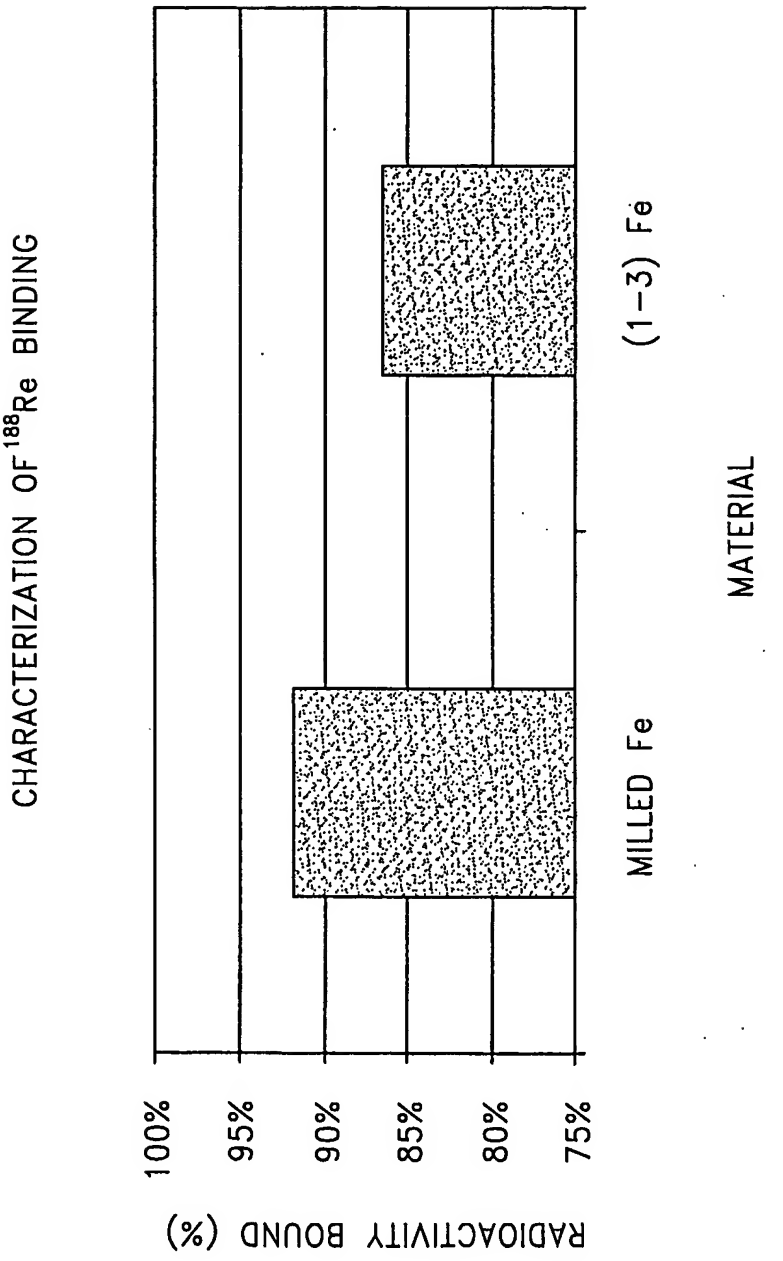
CHARACTERIZATION OF ^{188}Re BINDING
RADIOACTIVITY BOUND (%)
100%
95%
90%
85%
80%
75%
MILLED Fe
(1-3) Fe
MATERIAL

FIG. 3

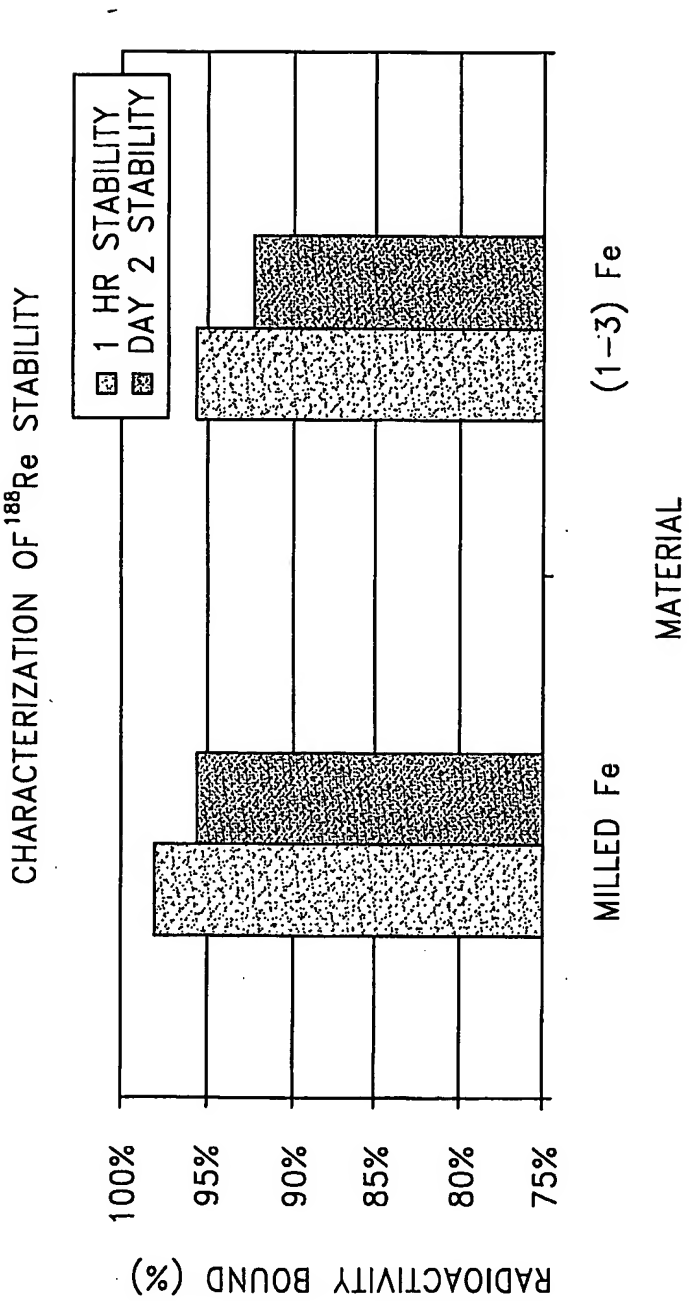
CHARACTERIZATION OF ^{188}Re STABILITY
1 HR STABILITY
DAY 2 STABILITY
RADIOACTIVITY BOUND (%)
100%
95%
90%
85%
80%
75%
MILLED Fe
(1-3) Fe
MATERIAL

FIG. 4

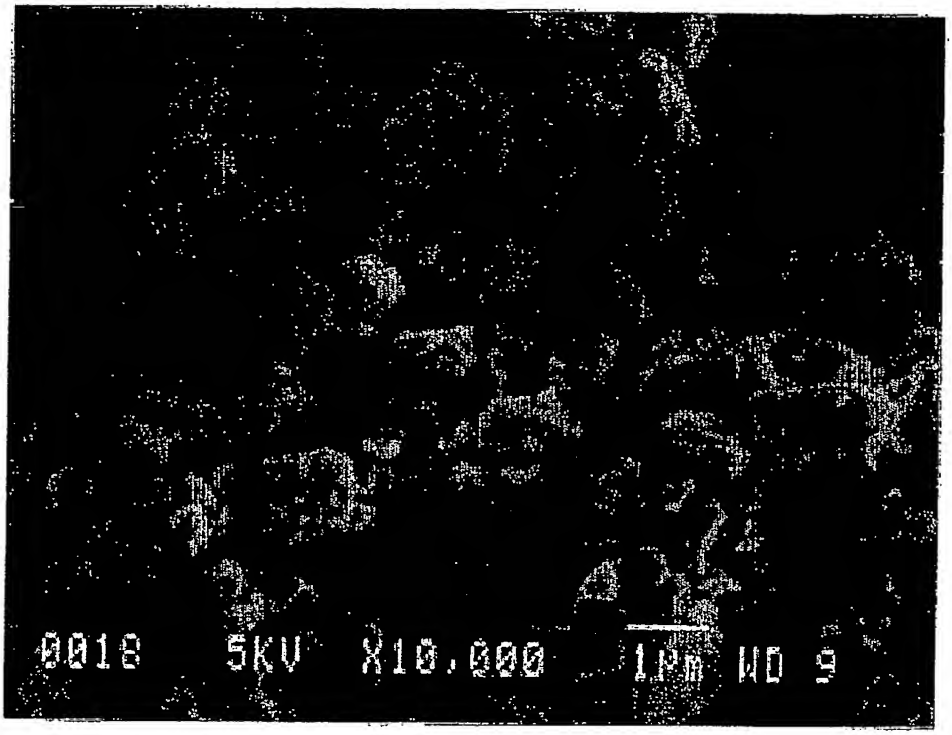

FIG. 5

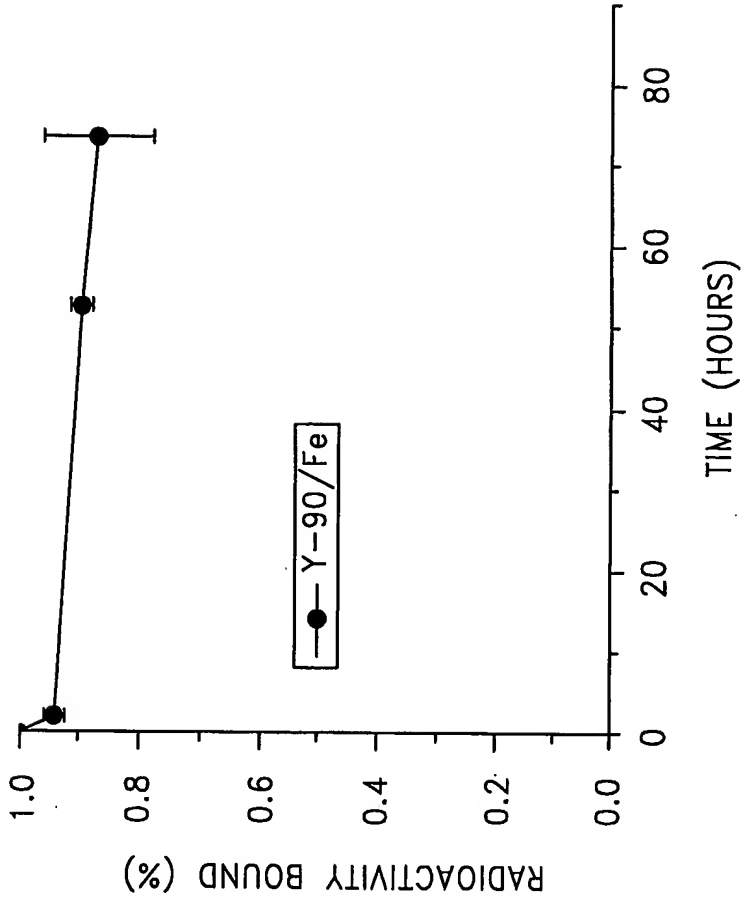
RADIOACTIVITY BOUND (%)
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
TIME (HOURS)
Y-90/Fe

FIG. 6

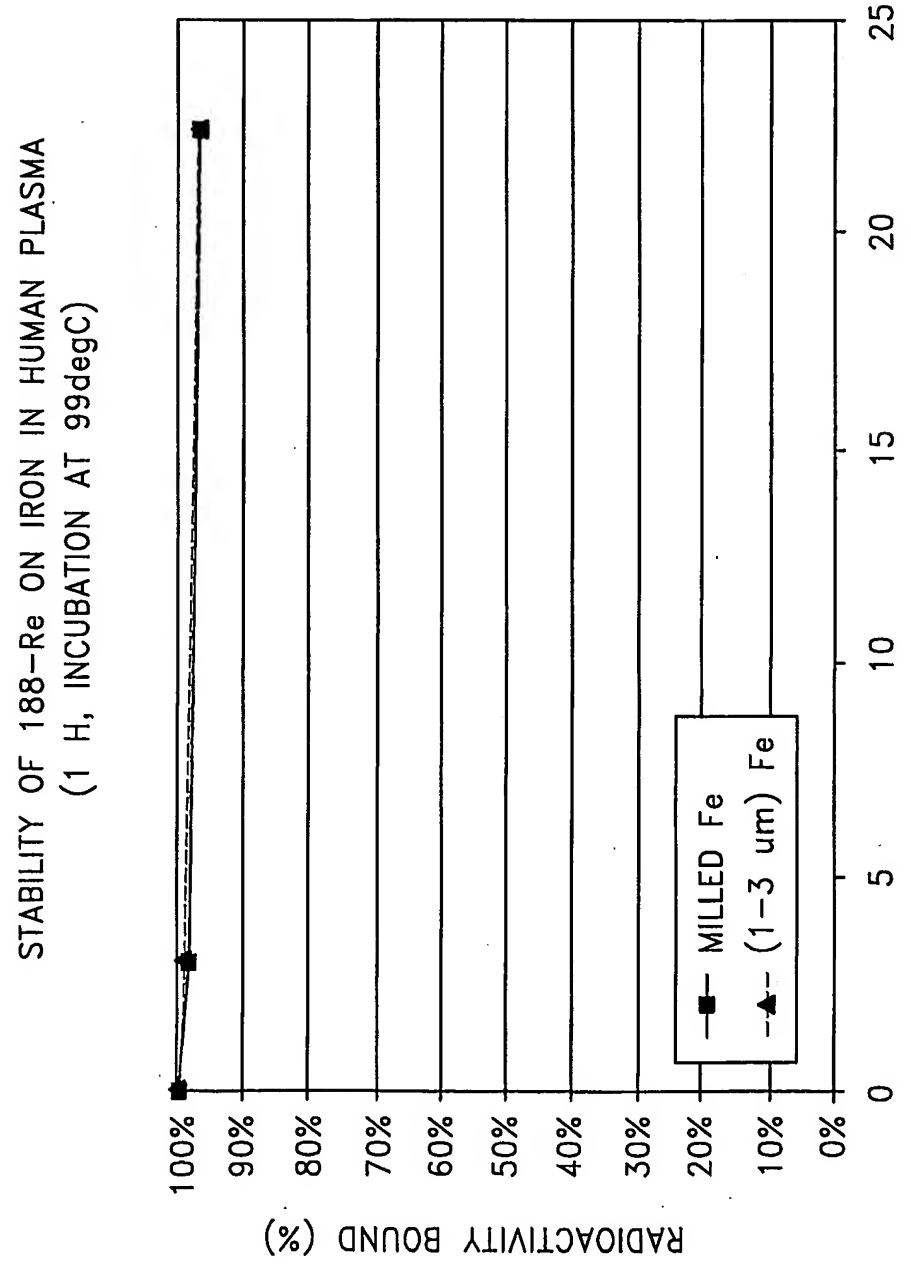
STABILITY OF 188-Re ON IRON IN HUMAN PLASMA
(1 H, INCUBATION AT 99degC)
RADIOACTIVITY BOUND (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
5
10
15
20
25
TIME (HOURS)
MILLED Fe
(1-3 um) Fe

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/00489

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 9/16, 9/50
US CL : 424/9.3, 9.32, 490
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/9.3, 9.32, 490

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,651,989 A (VOLKONSKY et al.) 29 July 1997 (29.07.1997), abstract, col. 7, lines 20-42, col. 8, lines 10-67.	1-3, 6, 10-12, 42, 59-61 --- 1-5, 7-9, 35-41
Y	US 4,345,588 A (WIDDER et al.) 24 August 1982 (24.08.1982), abstract, col. 9 - col. 14.	1-17, 35-62
Y	US 5,411,730 A (KIRPOTIN et al.) 02 May 1995 (02.05.1995), abstract, cols. 20-23.	
Y	US 5,122,418 A (NAKANE et al.) 16 June 1992 (16.06.1992), abstract, col. 19, lines 35-67; cols. 20-23; col. 33, lines 25-57; col. 34, lines 1-57.	1-17, 35-62

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
21 May 2003 (21.05.2003)

Date of mailing of the international search report
05 JUN 2003

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703)305-3230

Authorized officer
Sreenivasan Padmanabhan, PhD

Telephone No. 703-308-1123

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/00489

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim Nos.: 18-34
because they relate to subject matter not required to be searched by this Authority, namely:
Under the PCT Rule 39, the instant claimed are directed to non-statutory subject matter because they are directed to "Use of a composition" which are also not a statutory subject matter under 35 USC 101.

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claim Nos.: 31-34
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Continuation of B. FIELDS SEARCHED Item 3:
EAST, Medline, CAplus

Iron, magnetic, particle, 90Y, doxorubicin